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Contract No. NO1-NS-82388

QUARTERLY PROGRESS REPORT
October 1 - December 31, 1998

Report No. 4

"SAFE AND EFFECTIVE STIMULATION OF NEURAL TISSUE"

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ABSTRACT

In the last quarter, we have continued our evaluation of intracortical microstimulation in the cat's sensorimotor cortex using multi-electrode arrays. We investigated the neuronal changes resulting from various stimulation protocols using simultaneous or interleaved continuous stimulation. There was no instance of mechanically- or electrically-induced neuronal injury even with prolonged (4 day) or high frequency stimulation. Three 16-electrode (passive) arrays have been implanted into the cerebral cortex using a new electrode inserter. Histologic studies on these animals are in progress.

INTRODUCTION

In the past quarter, we have continued our investigations of the limits of safe stimulation of the cat's sensorimotor cortex using multi-electrode arrays of microelectrodes. In our QPR #2, we described the accumulation of lymphocytes around pulsed electrodes but did not describe the effects of the stimulation on the cortical neurons. In this report we have focused on the neuronal effects of microstimulation in some of the same animals, as well as in additional animals in which either the duration of the stimulation was extended or the sacrifice was delayed.

METHODS

Microelectrodes. Two types of microelectrode arrays were used in this series. The first contained 7 discrete activated iridium microelectrodes with shaft thickness of 35 μm and in which the geometric area of the exposed tips was 500 or 2,000 μm^2. Three longer shafts (50 μm in diameter) were added as inactive (uninsulated) stabilizing pins and also aided orientation during histologic evaluations. The second type of array

consisted of 16 microelectrodes having shaft diameters of 50 μm and varying in length from 1.1 mm (8) to 1.2 mm (8) with an additional 3 uninsulated stabilizing electrodes 2.5 mm in length and 75 μm in diameter. The electrode shafts taper to a final radius of curvature of approximately 6 μm. The array matrices were 2.5 mm in diameter and 0.75 mm in thickness.

Surgical Procedure. The 7-electrode arrays were implanted into 12 young adult cats of either sex, using general anesthesia and aseptic surgical technique. The animal's head was mounted in a stereotaxic holder, the scalp and muscles were reflected in a midline incision, and the pericruciate gyri (sensorimotor cortex) exposed. The frontal air sinus was partly filled with cranioplasty. The percutaneous connector was mounted to the skull with stainless steel screws and methacrylate bone cement. A macrostimulating electrode was placed on the dura over the postcruciate gyrus, and a recording electrode was implanted into the pyramidal tract through a small burr hold over the cerebellum. The large pyramidal tract potential evoked from the surface of the postcruciate gyrus was used to guide the recording electrode into the tract. The recording electrode was then sealed to the skull using methacrylate bone cement. A small dura flap, slightly larger than the array's superstructure (matrix), was made over the postcruciate cortex, and the arrays of 7 microelectrodes were inserted using a stereotaxically-mounted vacuum array holder.

In these animals, we did not suture the dura over the array, but in recent animals (IC-166 and thereafter, Table 1), we covered the array with a sheet of fascia taken from scalp musculature. The cortex was then covered with gelfoam and the bone flap replaced and sealed with cranioplasty.

New electrode inserter. Three 16-electrode arrays have been implanted in acute experiments in which we evaluated a new electrode inserter and the histologic effects of the insertion. The electrode inserter shown in Fig. 1 was designed to insert the electrodes precisely along their axis, in order to avoid damage to the electrodes and to avoid slashing the tissue. It inserts the array at a controlled velocity, to allow the microelectrodes to penetrate through the pia, but not rupture the microvasculature within the brain tissue. Prior to deployment, the microelectrode array is enclosed and

protected within the end of the barrel. A vacuum of approximately 300 mm of Hg holds the array against the end of a hollow sliding piece within the barrel. The distal end of the barrel is slotted to accommodate the electrical cable attached to the array.

The orifice at the end of the barrel is placed against the surface of the brain, so as to slightly depress the tissue. When the trigger is depressed the sliding air valve within the body of the instrument pushes forward a flexible steel wire, which extrudes the sliding piece and the array. The array, still held by the vacuum against the end of the slide, is pushed into the tissue at a predetermined velocity (0.5-2 m/sec). The speed of insertion is controlled by the spring compression adjustment screw, by the amount of vacuum ahead of the sliding air valve in the body of the instrument, and by a viscous-damping speed governor within the instrument's body. When the slide reaches the end of its travel, the air valve within the body of the instrument closes off the vacuum line. At the same instant, a valve near the end of the barrel opens and these two simultaneous events cause the vacuum within the barrel to collapse within a few ms, releasing the microelectrode array. The barrel is then retracted, leaving the array floating on the surface of the brain.

Stimulation Protocols. At least 45 days after implantation, the 7-hour test stimulation regimen was conducted, with the animal lightly anesthetized with Propofol. Before and after the test stimulation, the response evoked by the intracortical microelectrodes was recorded from the pyramidal tract. By this method, we have determined that the Propofol anesthesia does not reduce the amount of neural activity evoked by the microelectrodes and that it does not induce an elevation of the electrical threshold of the cortical neurons. Most stimulation regimens were 7 hours in duration, but in two animals (#'s 171 and 172) it was extended to 7 hrs/day on 4 days in succession to assess the effect of more prolonged stimulation on neurons and the accumulation of lymphocytes near the tips. The electrodes were pulsed continuously with charge-balanced, controlled-current, cathodic-first pulses. The pulse duration was 150 or 400 μsec/ph. In all cases, 5 microelectrodes were pulsed and two were left as unpulsed controls. To assess the role of charge density on neural damage and/or the lymphocytic accumulation, the exposed surface area was either 500 or 2,000 μm^2. In

most animals, the 5 microelectrodes were pulsed sequentially (interleaved) except for animal #'s 170 and 172, which were pulsed in the simultaneous mode. In one animal (IC-174) we pulsed all 5 electrodes simultaneously at a high frequency (500 Hz). The stimulus parameters for each animal are listed in Table 1. Most of the cats were sacrificed immediately after the stimulation for histologic evaluation of the electrode sites. Two animals, IC-167 and IC-174, were sacrificed at 48 hours after stimulation. In the case of the 16-electrode (unpulsed), acute implants, the animals were sacrificed 3-4 hours following implantation. The histologic results of the 16-electrode arrays are in progress and will be reported next quarter.

All surgical procedures are being recorded by videotape which also enables confirmation of the speed at which the arrays are inserted.

RESULTS

Table 1 lists the various electrical parameters and summarizes the histologic appearance of neurons near pulsed and unpulsed electrodes. Figs. 2A and 2B (IC-167) show the site of the tips of unpulsed and pulsed activated iridium microelectrodes. This animal was stimulated for 7 hours and sacrificed 2 days later. Note that few lymphocytes are present adjacent to the electrodes. A few glial cells are present at the sites of the pulsed and unpulsed tips; however, neurons as close as 15 μm to the tips appear normal.

Fig. 3 (IC-170, simultaneous stimulation) shows the site of the tip of an electrode pulsed for 7 hours/day on 4 successive days. The cat was sacrificed 45 minutes after cessation of the stimulation. Note the many normal-appearing neurons immediately adjacent to the site of the tip and the absence of both lymphocytes and glial elements. Fig. 4 (IC-172) is a second example of the effect of 7 hours of stimulation on 4 successive days. The tip site shows a few lymphocytes but mostly an accumulation of glial cells. All neurons in the vicinity appear to be normal.

TABLE I
INTRACORTICAL MICROSTIMULATION
NEURONAL STATUS

ANIMAL #	DURATION OF STIMULATION (HRS)	POST-STIM. TIME	STIMULATION PARAMETERS*					NEURONS NEAR THE TIP**						
			MODE	FREQ. (Hz)	CURRENT		CHARGE DENSITY (μC/cm²)	UNPULSED		PULSED				
					(μA)	(nC)								
166	7	45 min.	Interlv.	25	16	2.4	480	1 TBN	2 TBN	3 TBN	4 TBN	5 TBN	6 TBN	7 TBN
167	7	2 days	Interlv.	50	26.5	4.0	800	1 N	2 N	3 N	4 N	5 N	6 N	7 N
168	7	45 min.	Interlv.	50	26.5	4.0	800	2 N	4 N	1 N	3 N	5 N	6 N	7 N
169	7	4 days	Interlv.	50	26.5	4.0	800	3 N	4 OF	1 N	2 N	5 N	6 N	7 N
170	7 hrs/day 4 days	45 min.	Simult.	50	26.5	4.0	800	2 N	4 N	1 N	3 N	5 N	6 N	7 N
171	7	45 min.	Interlv.	50	26.5	4.0	200	4 N	5 N	1 N	2 N	3 N	6 N	7 N
172	7 hrs/day 4 days	45 min.	Simult.	50	26.5	4.0	200	1 N	7 N	2 N	3 N	4 N	5 N	6 N
173	7	45 min.	Interlv.	50	26.5	4.0	200	1 N	4 N	2 N	3 N	5 N	6 N	7 N
174	7	2 days	Interlv.	500	26.5	4.0	200	1 N	6 N	7 N	2 N	3 N	4 N	5 N
175	**Implanted 04/21/98; Stimulation Pending**													
176	**Implanted 10/08/98; Stimulation Pending**													
177	**Implanted 12/01/98; (16-electrode array pulsed on December 15)**													
178	**Implanted 12/09/98; Stimulation Pending**													
179	**Implanted 01/08/99; (Perfused on same day)**													
180	**Implanted 01/21/99; (Perfused on same day)**													

*Electrode tip surface areas were 500 μm² in animals 166-170; in 171-13 they were 2000 μm²
**Neuron code: N = Normal-appearing TBN = Electrode tip below neuronal layers OF = Occasional Flat

Fig. 5A (IC-174) shows an unpulsed tip site and 5B-F shows microelectrode sites that were pulsed at high frequency (500 Hz). Note the normal-appearing neurons, absence of lymphocytes and only occasional glial cells in the vicinity of the tips. There was no significant difference between the neurons surrounding pulsed and unpulsed tips. This was also true of neurons adjacent to the tip sites in animals IC-169, IC-171 and IC-173, in which only one of the 21 tip sites was associated with occasional, slightly flattened neurons (IC-169, #4; see Table 1). Thirteen of the remaining 20 electrode tracks showed slightly flattened neurons near the tracks at one or more levels. However, in no instance, whether along the tracks or at the tip sites, was there evidence of neuronal damage, which characteristically takes the form of dark, shrunken, stellate neurons with pericellular haloes.

DISCUSSION

Aside from slight mechanically-induced flattening of a few neurons near several of the tracks, the lack of any frank neuronal injury in this series which included prolonged (4-day) and high frequency (500 Hz, 26.5 μA, 4 nC/phase, 200 μC/cm^2) stimulation protocols, is very encouraging. The latter, in which 5 microelectrodes were pulsed simultaneously at 500 Hz and at a fairly high amplitude, was designed as a "worst-case" approximation of the paradigm in which the many microelectrodes comprising a dense array are pulsed in the interleaved mode, each at a much lower frequency. Also, the lack of injury to the neurons surrounding the electrodes pulsed at 500 Hz suggests that putative neural damage mechanisms that are related directly to the passage of the stimulus current (e.g., membrane electroporation) are unlikely to ever be a problem during stimulation at lower frequency, even when the stimulus is of fairly high amplitude (26.5 μA, 4 nC/phase, 200 μC/cm^2).

As reported in our previous QPRs #2 and #3, we have yet to determine the etiology of lymphocytic accumulation around the tips of the pulsed electrodes. However, the results reported here indicate that the lymphocytes disperse by 48 hours after the end of the stimulation and also when the stimulation is applied for several (e.g., 4) days. These observations are important with respect to long-term intracortical

microstimulation (as in a clinical neural prosthesis). The dispersion of the lymphocytes makes possible a more complete histologic appraisal of neurons, since when the lymphocytes are present in large numbers, they visually obscure the neurons and make their histologic evaluation difficult.

Histologic evaluation of the 16-electrode arrays implanted with the new inserter is in process.

WORK NEXT QUARTER

To further investigate the phenomenon of lymphocyte accumulation at the tips of pulsed microelectrodes, we have initiated *in vitro* stimulations of incubated human lymphocytes. The lymphocytes will be stimulated with and without the presence of glial cells and with salt bridge electrodes as well as faradaic stimulating electrodes to determine if there exists a relationship between electrochemically-generated products and lymphocytic accumulation.

We will also continue evaluation of 19-electrode passive implants in the sensorimotor cortex.

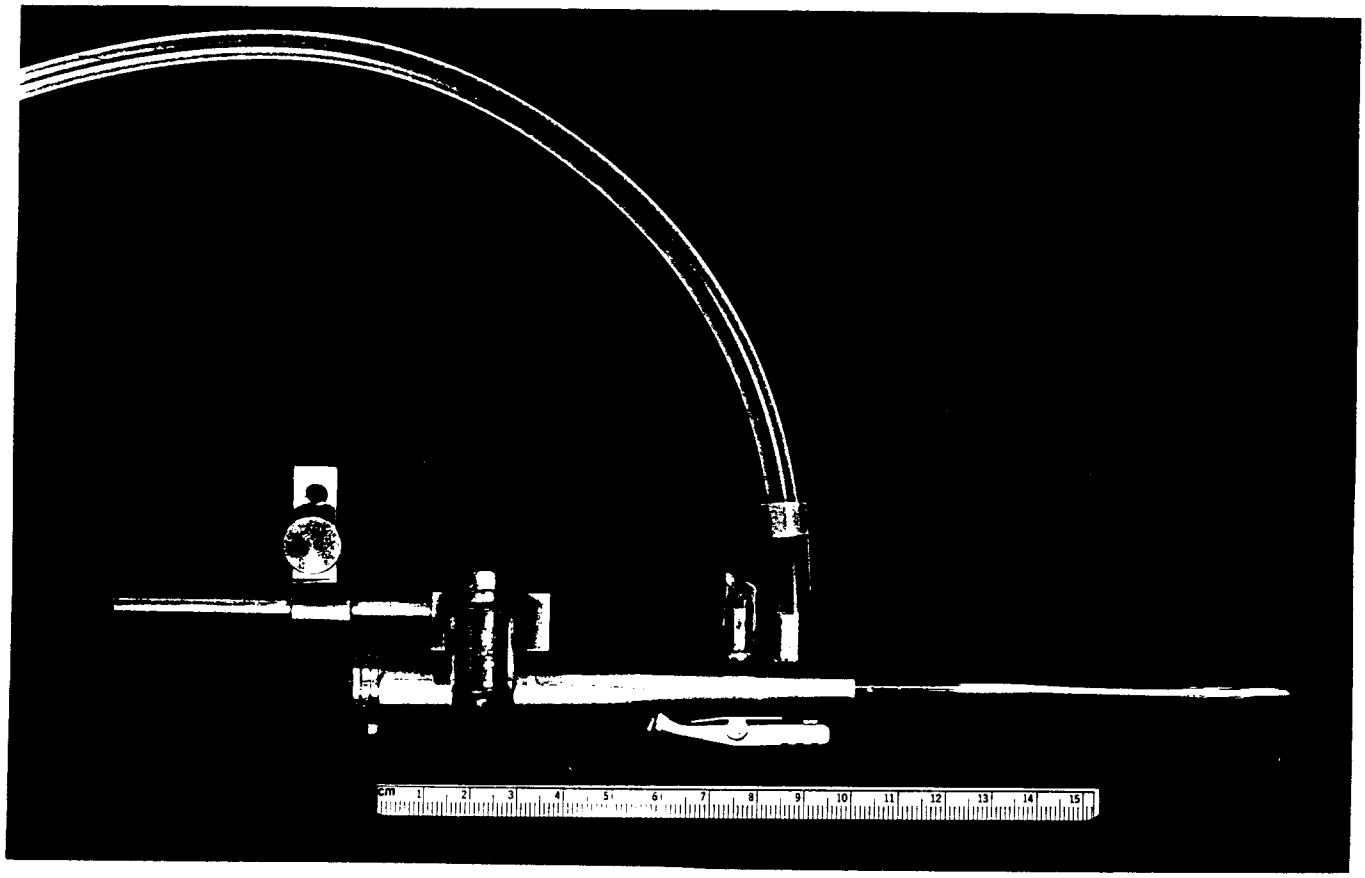


FIG. 1

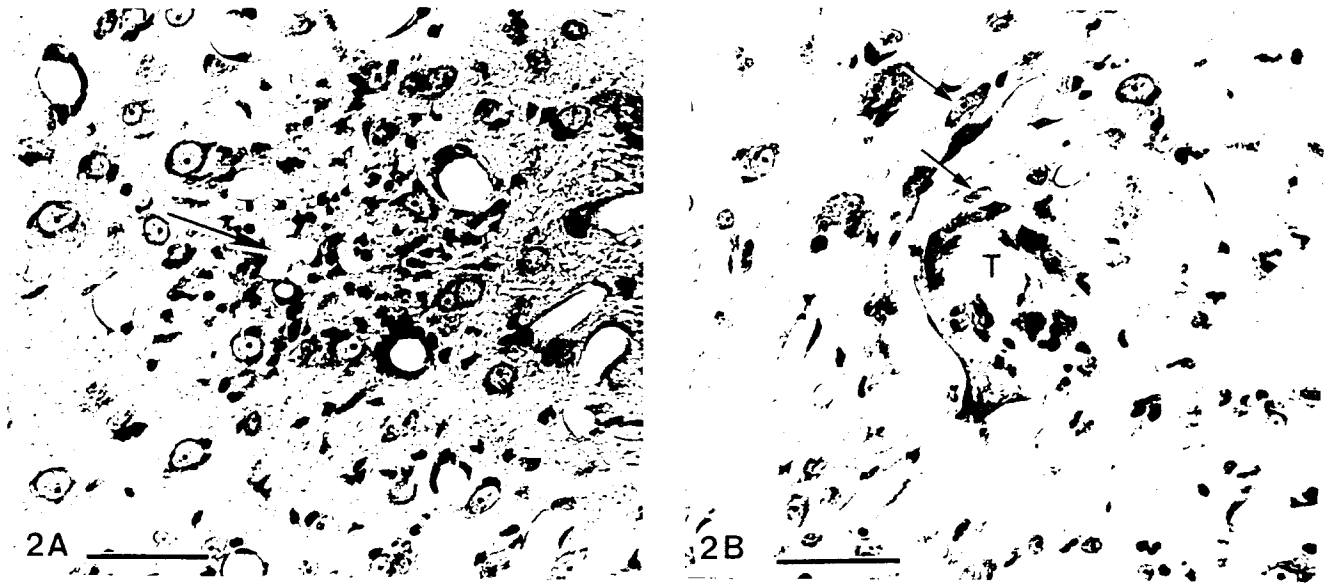


Fig. 2A. IC-167. Horizontal section of unpulsed #2 electrode tip site (arrow) three months after implantation. A few lymphocytes (small, dark, round profiles) and glial elements accompany the track. All neurons in the vicinity appear normal. H&E stain. This and all subsequent micrographs are from horizontally cut paraffin sections through the left cruciate gyri and stained with Nissl or H&E. The bar in each micrograph represents 50 µm.
Fig. 2B. IC-167. Electrode #7, tip site (T), pulsed 7 hours per day for 2 days and perfused 45 minutes later. A few glial cells are seen at the tip site although lymphocytes are not present. Aside from an occasional flat neuron (arrow), all others appear normal. Nissl stain.

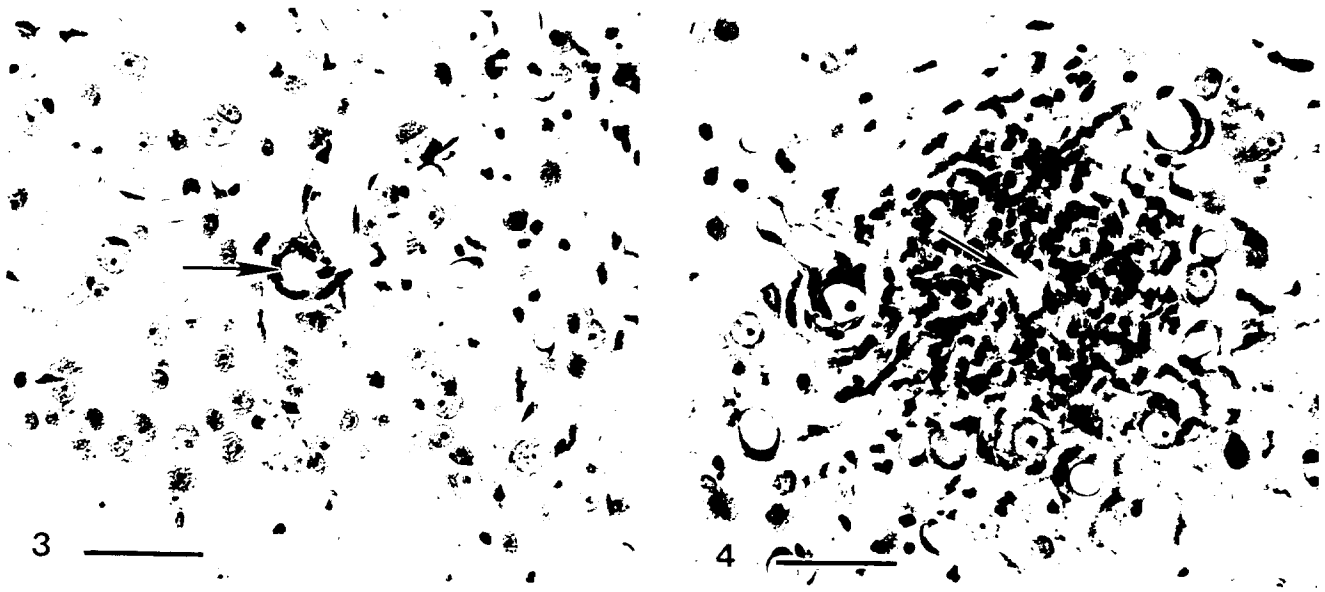
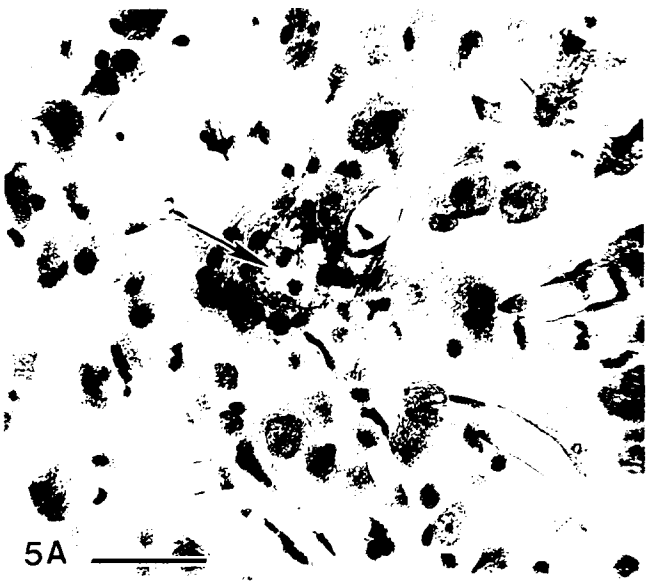


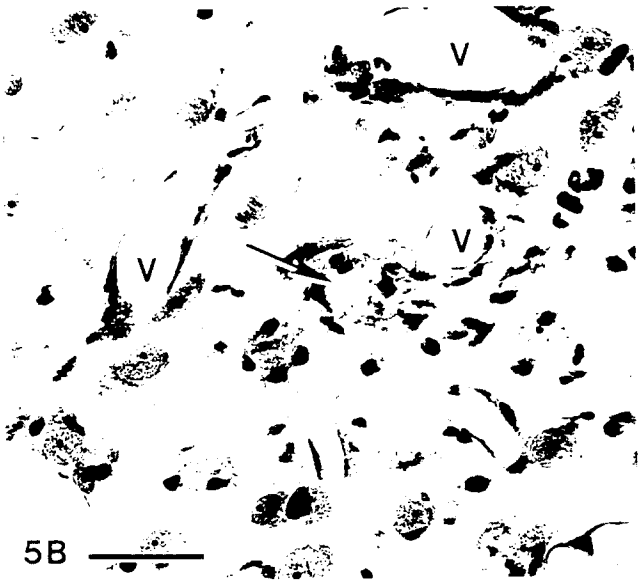
Fig. 3. IC-170. H&E-stained section through the tip site (arrow) pulsed 7 hours per day for 4 days and perfused 45 minutes later. Neither glial cells nor lymphocytes are associated with the tip site. Though the neurons do not appear distinctive in this and other H&E sections, they do appear normal.
Fig. 4. IC-172. Tip site (arrow) of Electrode #3, pulsed for 4 days at 7 hours per day and sacrificed 45 minutes later. Numerous glial cells but only a few lymphocytes surround the track. All neurons in the field appear normal. Nissl stain.

Fig. 5A. IC-174. H&E-stained, unpulsed #6 electrode tip site (arrow) 6 months after implantation. Lymphocytes are absent and only occasional glial cells are adjacent to the tip site. All neurons appear normal. H&E stain.

Figs. 5B-F. IC-174. All electrode tip sites were pulsed for 7 hours and perfusion-fixed 2 days later. **B.** Tip site #2 (arrow) showing a few adjacent glial elements. Lymphocytes are not present. Nearby blood vessels (V) show hypertrophy. All neurons appear normal including some within 15 μm. H&E stain. **C.** H&E-stained #3 tip site (T) shows several glial cells and occasional lymphocytes. All neurons appear nromal. **D,E,F.** Nissl-stained tip sites #4, 5 and 6, respectively. Aside from marked vascular hypertrophy (V) near tip site #6 (Fig. 5F), the findings at all tip sites are essentially the same, namely: complete absence of lymphocytes, normal-appearing neurons and relatively few glial cells.



5A



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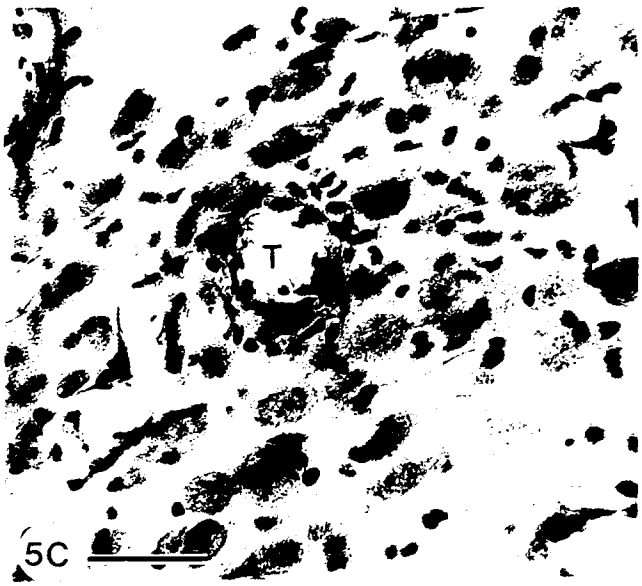

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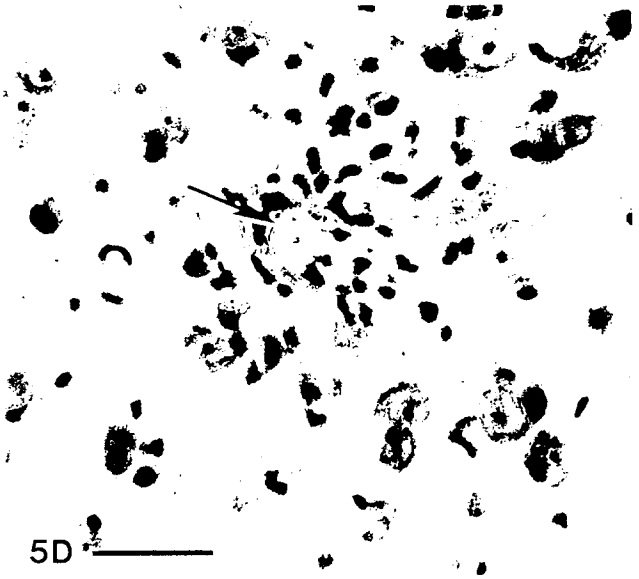
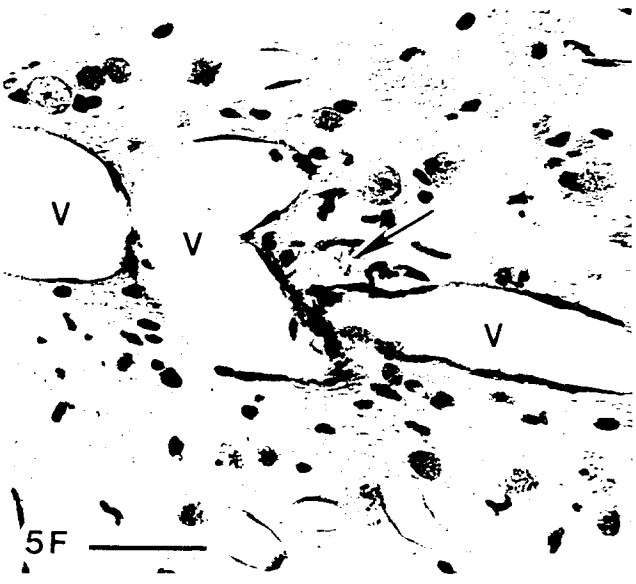


5D

5E



5F